

Potassium-induced alleviation of salinity stress in *Brassica campestris* L.

Research Article

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Abstract: Salinity is an important abiotic factor that adversely affects major agricultural soils of the world and hence limits crop productivity. An optimum mineral-nutrient status of plants plays critical role in determining plant tolerance to various stresses. A pot experiment was conducted on mustard (*Brassica campestris* L.) to study the protective role of added potassium (K, 40 mg kg⁻¹ soil) against salinity-stress (0, 40 and 80 mM NaCl)-induced changes in plant growth, photosynthetic traits, ion accumulation, oxidative stress, enzymatic antioxidants and non-enzymatic antioxidants at 30 days after sowing. Increasing NaCl levels decreased the growth, photosynthetic traits and the leaf ascorbate and glutathione content but increased the leaf ion accumulation and oxidative stress, and the activity of antioxidant enzymes. In contrast, K-nutrition improved plant growth, photosynthetic traits, activity of antioxidant enzymes and the ascorbate and glutathione content, and reduced ion accumulation and oxidative stress traits in the leaves, more appreciably at 40 mM than at 80 mM NaCl. The study illustrates the physiological and biochemical basis of K-nutrition-induced NaCl tolerance in mustard as a means to achieving increased crop productivity in a sustainable way.

Keywords: Antioxidant defence system • *Brassica campestris* • Ion accumulation • Oxidative stress • Plant growth • Potassium nutrition • Soil-salinity

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Abbreviations

APX - ascorbate peroxidase;
AsA - ascorbate (reduced);
DAS - days after sowing;
DHA - dehydroascorbate;
DTNB - 5',5'-dithio-bis-2-nitro-benzoic acid;
DTT - 1,4-dithiothreitol;
EDTA - ethylenediaminetetraacetic acid;
FW - fresh weight;
GR - glutathione reductase;
GSH - glutathione (reduced);
GSSG - glutathione (oxidized);
LA - leaf area;
MBTH - 3-methyl-2-benzothiazoline hydrazone;
NBT - nitroblue tetrazolium;
NEM - N'-N-ethylemaleimide;

P_N - net photosynthetic rate;
ROS - reactive oxygen species;
SOD - superoxide dismutase;
TBARS - thiobarbituric acid reactive substances.

1. Introduction

Salt affected soils are an integral part of the landscape in arid and semiarid tropical and/or non-tropical regions. It is estimated that approximately 831 million hectare (mha) are affected world wide, which accounts for more than 6% of the world's land surface area [1]. Plants growing in salt affected soils are exposed to a number of unfavourable conditions which include low soil water potential, nutrient imbalance and excessive Na⁺ and Cl⁻ accumulation and/or K⁺ deficiency in cells [2-4]. The cumulative effects

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of all the above factors adversely affect physiological and biochemical processes of plants leading to poor growth and yield [5,6]. Oxidative damage at the cellular/organ level under adverse conditions including salt stress resulting from the imbalance between production of reactive oxygen species (ROS) and antioxidant defence is often studied to understand the mechanisms of injury [4,7]. ROS are highly reactive and cause damage to major cellular components [8]. Plant tolerance to salinity stress may be due to ion homeostasis, osmotic adjustment and efficient and synchronous action of various enzymatic and non-enzymatic components of the antioxidant defence system [6]. A level of nutrients considered being sufficient for plant growth under non-stress conditions may or may not be sufficient under salt stress. Where plant growth is also adversely affected because of higher nutrient requirement under salt stress, increased supply of nutrients is likely to benefit plants under stress [9,10]. Grattan and Grieve [11] have extensively reviewed the impact of increased nutrient supply on horticultural crops under salt stress.

The macronutrient potassium (K) plays an important role in stimulation of root growth, increasing leaf area, chlorophyll content, net assimilation rate, balancing membrane potential and turgor, activating enzymes, regulating osmotic pressure, stomatal movement and tropisms and reduction of the excess uptake and translocation of ions such as Na^+ and Cl^- [12,13]. The antagonistic effect of Na^+ (the predominant cation in salt affected soil) on uptake of K is well established. Externally supplied K may also increase endogenous K^+ content of seeds, required to tolerate salinity at germination [14], and ameliorate abiotic-stress effects [13]. However, there is limited understanding of the mechanisms associated with K alleviating the adverse effects of salt stress accrued oxidative stress in plants.

The present study is aimed at studying the effects of added K (through nutrient solution) on photosynthetic functions, reducing equivalents (AsA-GSH) and control of the coordination of enzymatic (SOD, APX, GR) and non-enzymatic (AsA, GSH) in pot culture raised-*Brassica campestris* plants exposed to varying salinity levels. The outcome of the study may be helpful in formulating a protocol for the application of a proper level of K to NaCl-affected soil which consequently may be helpful in achieving increased crop productivity in a sustainable way.

2. Experimental Procedures

2.1 Plant material and growth conditions

Seedlings of mustard (*Brassica campestris* L. cv. Pusa Gold) were raised from seeds in 40-cm-diameter plastic

pots filled with 8 kg soil. The experimental soil had the following characteristics: texture - loamy sand (83.6% sand, 6.8% silt and 9.6% clay), pH - 7.1, E.C. - 0.23 dsm^{-1} , organic C - 0.28%, available P - 14 kg ha^{-1} , available K - 158 kg ha^{-1} [15]. The experiment was carried out during winter under natural day/night [photosynthetically active radiation (PAR) $>900 \mu\text{mol (photon) m}^{-2} \text{ s}^{-1}$, temperature $22\pm3^\circ\text{C}$; relative humidity (RH) 62–72% in the Department of Botany, Hamdard University, New Delhi, India (28.380° N, 77.110° E and 228 m altitude). Two plants per pot were grown with Hoagland's nutrient solution [16] and were fed with 250 ml of nutrient solution with 0 (control), 40, 80 mM NaCl solution (i.e. 0.001, 5.0, and 8.0 dS m^{-1} , respectively) every alternate day and 200 ml of deionized water daily. Extra K (KCl, Analytical grade) was applied at 40 mg kg^{-1} soil once at the start of the study. The pots were arranged in a randomized block design and replicated three times. All the measurements were performed at 30 d after sowing (DAS).

2.2 Measurements of growth and photosynthetic traits

Plant dry mass (PDM) was recorded after drying the plants for 48 h to a constant weight at 80°C . Leaf area (LA) was measured by a leaf area meter (Licor 300 model). Net photosynthetic rate (P_n), stomatal conductance (gs) and intercellular CO_2 concentration (C_i) were measured on a fully expanded third leaf from the top of the main shoot, using an infra red gas analyzer (Licor 6400 model) at 9:00–11:00 am at saturating light intensity.

2.3 Oxidative stress traits

2.3.1 Thiobarbituric acid reactive substances content

For measuring lipid peroxidation, the content of thiobarbituric acid reactive substances (TBARS) was measured by the method of Cakmak and Horst [17], recording the absorbance at 532 nm and correcting it for non-specific turbidity by subtracting the absorbance at 600 nm. The TBARS content was calculated using the extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

2.3.2 H_2O_2 content

H_2O_2 assay was done according to Okuda *et al.* [18]. Fresh leaf (0.5 g) was homogenized in 2.0 ml of pre-chilled HClO_4 (0.2 N), and the homogenate was centrifuged at $20,000\times g$ for 5 min. To remove HClO_4 , the supernatant was neutralized to pH 7.5 with KOH (4 N), followed by centrifugation at $1,000\times g$ for 1 min. Ten units of catalase were added to an aliquot, and the mixture was incubated for 15 min at 25°C . The reaction was stopped by boiling for 1 min. The reaction mixture (1.5 ml) contained 1.0 ml of aliquot, 400 μl of 3-(dimethylamino) benzoic acid (DMAB, 12.5 mM)

prepared in phosphate buffer (0.375 M, pH 6.5), 80 μ l of 3-methyl-2-benzothiazoline hydrazone (MBTH) and 20 μ l of peroxidase (0.25 unit). The reaction was started by the addition of peroxidase, and the increase in absorbance was recorded at 590 nm.

2.4 Components of the antioxidant defence system

2.4.1 Contents of non-enzymatic components - reduced ascorbate and glutathione

The content of reduced (GSH) glutathione was estimated following the method of Anderson [19]. Fresh leaf (0.5 g) was homogenized in 2.0 ml of 5% (w/v) sulphosalicylic acid at 4°C. The homogenate was centrifuged at 10,000 \times g for 10 min. To 0.5 ml of supernatant, 0.6 ml of K-phosphate buffer (100 mM, pH 7.0) and 40 μ l of 5' 5' dithiobis-2-nitrobenzoic acid (DTNB) were added and absorbance was recorded after 2 min at 412 nm on a UV-VIS spectrophotometer (Lambda Bio 20, Perkin Elmer, MA, USA).

The content of reduced (AsA) ascorbate was determined by the method of Law *et al.* [20]. Fresh leaf (0.5 g) was homogenized in 2.0 ml of K-phosphate buffer (100 mM, pH 7.0) containing 1 mM EDTA and centrifuged at 10,000 \times g for 10 min. To 1.0 ml of supernatant, 0.5 ml of 10% (w/v) trichloroacetic acid (TCA) was added, thoroughly mixed and incubated for 5 min at 4°C. To 1.5 ml of the above solution, 0.5 ml of NaOH (0.1 M) was mixed and centrifuged at 5,000 \times g for 10 min at 20°C. The aliquot thus obtained was equally distributed into two separate microfuge tubes (750 μ l each). For estimation of AsA, 200 μ l of K-phosphate buffer (150 mM, pH 7.4) was added to 750 μ l of aliquot. For DHA estimation, 750 μ l of aliquot was added to 100 μ l of 1,4-dithiothreitol (DTT) followed by vortex-mixing, incubation for 15 min at 20°C and addition of 100 μ l of 0.5% (w/v) NEM. Both the microfuge tubes were then incubated for 30 s at room temperature. To each sample tube, 400 μ l of 10% (w/v) TCA, 400 μ l of H₃PO₄, 400 μ l of 4% (w/v) bipyridyl dye (N,N-dimethyl bipyridyl) and 200 μ l of 3% (w/v) FeCl₃ were added and thoroughly mixed. Absorbance was recorded at 525 nm after incubation for 1 h at 37°C.

2.4.2 Assays of enzymatic components

Fresh leaf (0.5 g) was homogenized in K-phosphate buffer (100 mM, pH 7.0) containing 0.5% (v/v) Triton X-100 and 1% (w/v) polyvinylpyrrolidone (PVP) in a pre-chilled mortar and pestle. The homogenate was centrifuged at 15,000 \times g for 20 min at 4°C. The supernatant was used for the enzyme assays. For APX, the homogenizing buffer was supplemented with 2 mM ascorbate.

Superoxide dismutase (SOD) activity was assayed by monitoring the inhibition of photochemical reduction

of nitroblue tetrazolium (NBT), according to Dhindsa *et al.* [21]. One unit of SOD was defined as the amount of enzyme required to cause 50% inhibition of the NBT reaction.

Activity of ascorbate peroxidase (APX) was determined according to Nakano and Asada [22] and calculated by using extinction coefficient 2.8 mM⁻¹cm⁻¹. One unit of enzyme was the amount necessary to decompose 1.0 μ mol of the substrate min⁻¹ at 25°C.

Glutathione reductase (GR) activity was determined, following Foyer and Halliwell [23], by monitoring the GSH-dependent oxidation of NADPH. It was calculated by using the extinction coefficient 6.2 mM⁻¹cm⁻¹. One unit of enzyme was the amount necessary to decompose 1.0 μ mol of NADPH min⁻¹ at 25°C.

2.5 Na⁺, Cl⁻ and K⁺ contents

Na⁺ and Cl⁻ content was determined in digested plant samples as described by Khan *et al.* [6] using Tri acid mixture (TAM). TAM is a mixture of nitric acid, sulphuric acid and perchloric acid (10:5:4). Na⁺ content in the digested samples of root and leaf was estimated using a flame photometer (2273, Khera, New Delhi, India) and values were compared with a standard curve prepared from NaCl. The content of Cl⁻ in digested root and leaf samples was estimated by titration against 0.02 N silver nitrate solution using 5% K₂CrO₄ as indicator.

K⁺ was estimated by the method of Lindner [24]. Potassium (K) was analyzed in the digested sample using flame photometry. The instrument was adjusted to zero (0 ppm), 25, 50 and 100 ppm readings. The readings were recorded directly in ppm and converted to actual values of K in plant samples by multiplying by a dilution factor and expressed as %.

2.6 Statistical analysis

The data were analysed statistically and standard errors calculated. Analysis of variance (ANOVA) was performed using SPSS (10.0 Inc., USA). The presented mean values were separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

3. Results

3.1 Growth and photosynthetic traits

Increasing salinity stress had an inhibitory effect on plant dry mass, leaf area, net photosynthesis, stomatal conductance and intercellular CO₂ concentration. These variables declined by 21.47%, 15.0%, 13.0%, 33.3% and 8.52%, at 40 mM NaCl and by 42.9%, 66.4%, 37.0%, 51.2% and 42.6%, at 80 mM NaCl in comparison with respective values in the control

(0 mM NaCl). Supply of K (40 mg kg⁻¹ soil) maximally mitigated the 40 mM NaCl-caused decrease in plant dry mass, leaf area and photosynthetic traits. Compared with controls, only 5.0%, 6.6%, 1.3%, 7.6% and 2.8% increase was noticed in plant dry mass, leaf area, net photosynthesis, stomatal conductance and intercellular CO₂ concentration respectively (Figure 1).

3.2 Oxidative stress traits and Na⁺, Cl⁻ and K⁺

Figure 2 shows that the level of lipid peroxidation, in terms of TBARS content, increased with an increase in the degree of soil salinity. Soil NaCl (80 mM) caused a significantly high lipid peroxidation and H₂O₂ generation. The contents of TBARS, H₂O₂, Na⁺ and Cl⁻ increased by 116.3%, 150.5%, 129.3% and 51.8% respectively, due to application of 40 mM NaCl, compared with the control. These variables increased by 365.2%, 531.3%, 219.0% and 98.4% respectively, at 80 mM NaCl salinity. However, addition of K (40 mg K kg⁻¹ soil) completely alleviated the adverse effects of 40 mM NaCl salinity in terms of increased TBARS, H₂O₂, Na⁺ and Cl⁻ contents. Even at 80 mM NaCl, K fertilisation significantly mitigated the adverse effects of salinity stress on these parameters. The K⁺ content was decreased by 14.1% and 21.1% under the influence of 40 and 80 mM NaCl, respectively, compared with the control. Whereas, the addition of K improved K content at both levels of salinity. The maximum (3.8%) increase was observed when 40 mg K kg⁻¹ soil was supplied (Figure 2).

3.3 Non-enzymatic and enzymatic components of the antioxidant defence system

AsA and GSH content decreased by 16.6% and 20.5% in plants exposed to 40 mM NaCl and by 30.1% and 67.9% at 80 mM NaCl. However, at 40 mM NaCl, added K helped to reverse this decrease and values reached parity with those in plants at 0 mM NaCl (Table 1). At

80 mM NaCl, K application was effective in reducing the adverse effects of salinity on AsA and GSH.

The activities of SOD, APX and GR enzymes were increased over the control in response to salt stress. These increases were 29.2, 62.6 and 16.6% respectively at 40 mM NaCl, and 45.1, 79.8 and 92.2% at 80 mM NaCl. The addition of K to plants grown at 40 mM NaCl showed a tendency to achieve the SOD level of unstressed plants (0 mM NaCl), with a 13.2% increase. This was also true with plants at 80 mM NaCl with added K. In contrast, there was a further increase in the activity of APX and GR in response to added K both at 40 mM and 80 mM NaCl. There was a 74.4% and 85.5% increase, respectively, in APS and GR activities at 40 mM NaCl compared with the control. When 80 mM NaCl treated plants were supplemented with the same K dose the same trend of increase in activity of these enzymes was observed over the control. The increase in APX and GR was 85.4% and 96.4% respectively (Table 2).

Treatments	AsA content (nmol g ⁻¹ FW)	GSH content (nmol g ⁻¹ FW)
Control	186 ^d ± 22.32	312 ^a ± 37.44
40 mM NaCl	155 ⁱ ± 18.60 (-16.67)	248 ^b ± 29.76 (-20.51)
40 mM NaCl + 40 mg K kg ⁻¹ soil	191 ^c ± 22.92 (2.69)	318 ^a ± 38.16 (1.92)
80 mM NaCl	130 ^g ± 15.60 (-30.11)	100 ^h ± 12.00 (-67.95)
80 mM NaCl + 40 mg K kg ⁻¹ soil	162 ^e ± 19.44 (-12.90)	129 ^g ± 15.48 (-58.65)

Table 1. Contents of ascorbate (AsA) and glutathione (GSH) in *Brassica campestris* L. leaves as influenced by NaCl stress and K nutrition at 30 d after sowing. Values [means ± SE (n=3)] in parenthesis show percent variation over the control and the values showing the same letter within the same column are not significantly different at P≤0.05, according to Duncan's Multiple Range Test. FW = Fresh weight.

Treatments	SOD activity (mg ⁻¹ protein m ⁻¹)	APX activity (mg ⁻¹ protein m ⁻¹)	GR activity (mg ⁻¹ protein m ⁻¹)
Control	5.10 ^d ± 0.40	0.541 ⁱ ± 0.04	0.160 ^k ± 0.011
40 mM NaCl	7.21 ^e ± 0.57 (29.26)	1.45 ⁱ ± 0.11 (62.69)	0.192 ^k ± 0.013 (16.67)
40 mM NaCl + 40 mg K kg ⁻¹ soil	5.88 ^d ± 0.47 (13.27)	2.12 ^h ± 0.16 (74.48)	2.11 ^h ± 0.14 (85.59)
80 mM NaCl	9.30 ^a ± 0.83 (45.16)	2.69 ⁱ ± 0.21 (79.89)	2.35 ^g ± 0.16 (92.20)
80 mM NaCl + 40 mg K kg ⁻¹ soil	8.55 ^b ± 0.68 (40.35)	3.71 ^e ± 0.29 (85.42)	2.88 ^f ± 0.20 (96.53)

Table 2. Activity of superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR) in *Brassica campestris* L. leaves as influenced by NaCl stress and K nutrition at 30 d after sowing. Values [means ± SE (n=3)] in parenthesis show percent variation over the control and the values showing the same letter within the same column are not significantly different at P≤0.05, according to Duncan's Multiple Range Test.

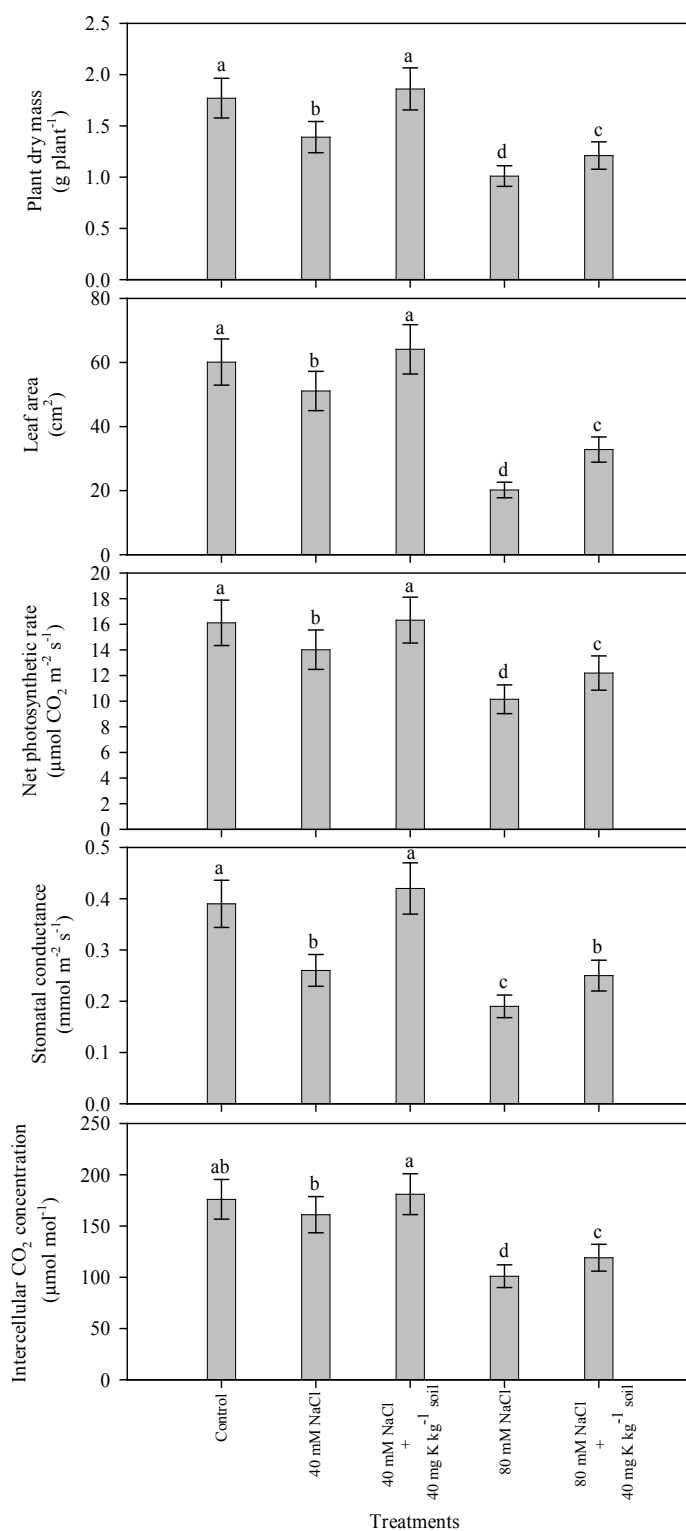


Figure 1. Plant dry mass, leaf area, net photosynthetic rate, stomatal conductance and intercellular CO₂ concentration in *Brassica campestris* L. as influenced by NaCl stress and K nutrition at 30 d after sowing. Values are means \pm SE ($n=3$). Bars showing the same letter are not significantly different at $P \leq 0.05$, according to Duncan's Multiple Range Test.

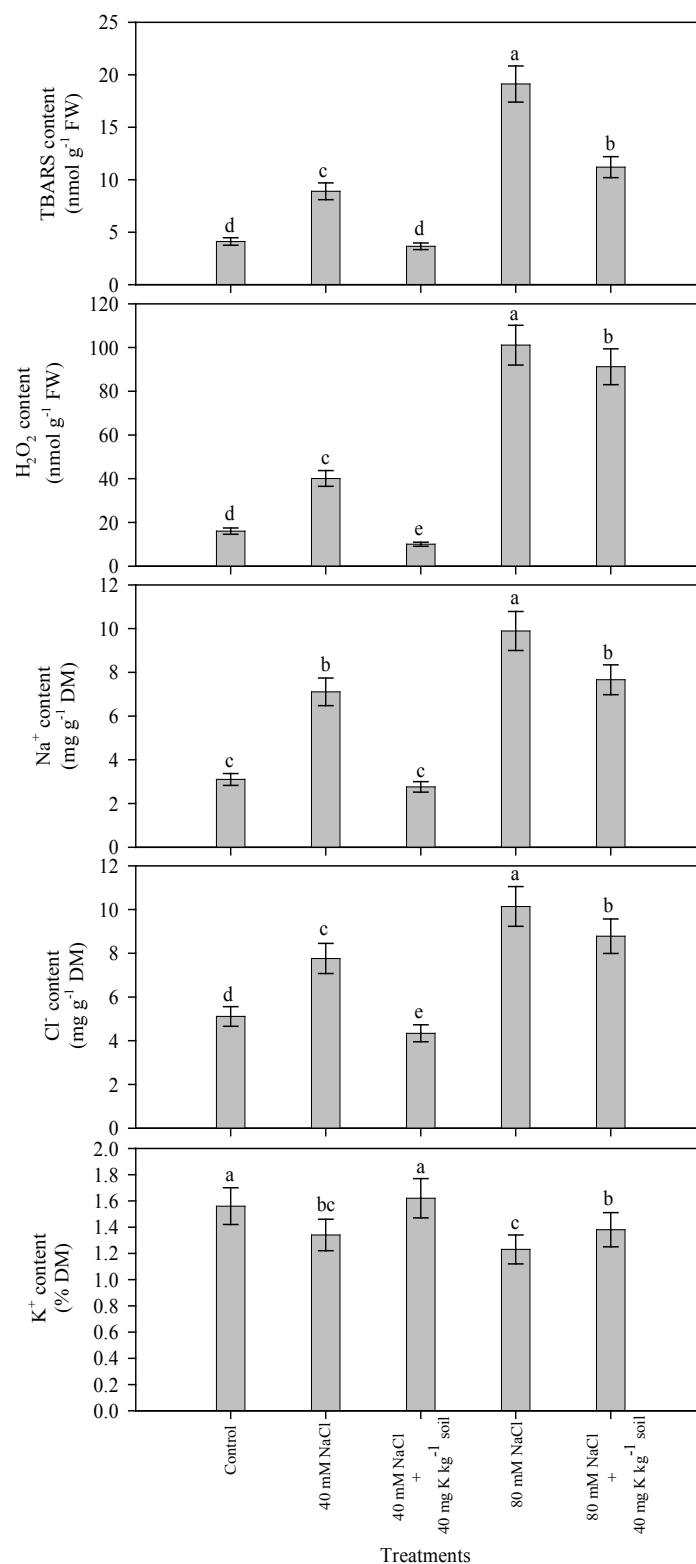


Figure 2. TBARS, H_2O_2 , Na^+ , Cl^- and K^+ contents in *Brassica campestris* L. leaves as influenced by NaCl stress and K nutrition at 30 d after sowing. Values are means \pm SE ($n=3$). Bars showing the same letter are not significantly different at $P \leq 0.05$, according to Duncan's Multiple Range Test.

4. Discussion

The adverse effect of increased soil-salinity on plant growth and development has been widely documented [2,6,25]. Growth and development are an outcome of coordination of the main biological processes in plants [26]. Availability of nutrients and the presence of stressors influence overall growth performance. Nutrient availability can be positively correlated whereas stress conditions are often negatively correlated to growth phenomena [27].

In the present study, increase in soil salinity caused a significant decline in leaf area and plant dry mass. Leaf area is considered a sensitive measure of effect in various salt-stressed plants and is also regarded as a major factor responsible for decreased dry mass of the affected individuals [25,28]. Reduction in leaf area expansion due to salinity stress has been observed in pepper [29,30] and strawberry [31]. The decrease in leaf area is attributed to decreased leaf cellular expansion and/or to physiological disorders triggered by salt stress, accumulation of Na^+ and Cl^- in different plant tissues and by reduction of net photosynthesis rate and chlorophyll content in leaf tissue [27,28]. Reduction in leaf area means reduction in size of the solar panel to harvest solar energy [32]. Even if the rate of photosynthesis remains unaffected under stress, decrease in leaf area itself is likely to have a negative impact on total dry matter production. Potassium application (40 mg K kg^{-1} soil) mitigated the adverse effects of salinity stress and improved the leaf area and plant dry mass significantly (Figure 1). K nutrition restored the NaCl-induced decline in the K^+ and Ca^{2+} content in plants and might help in improving the growth of stressed plants in terms of leaf area, leaf dry mass and plant dry mass. Our findings are in agreement with those of Arshi *et al.* [28] and Elkhatab *et al.* [33].

Salinity stress (40 and 80 mM NaCl) severely affected the photosynthetic traits such as net photosynthetic rate (P_n), stomatal conductance (gs) and intercellular CO_2 concentration (Ci). This agrees with the finding of others in various plant species [5,34]. Added K improved these traits considerably in the present study. High K concentrations in the stroma are reported to be necessary to maintain optimum photosynthetic capacity under stress conditions [35]. Added K at both the salinity levels (40 and 80 mM) significantly improved stomatal conductance, net photosynthesis, leaf area and plant dry weight. The values for these attributes in plants exposed to 40 mM NaCl were at par with those of unstressed plants (0 mM NaCl), as if the plants were not experiencing any stress. However, at 80 mM NaCl, K application did

little to towards bringing parity with the control level (0 mM NaCl). Salt stress resulted in a decrease in intracellular CO_2 concentration particularly at 80 mM NaCl, and K fertilization did help to improve this. Like other attributes mentioned above, it was at parity with the control (0 mM NaCl) but not significantly different from the value at 40 mM NaCl. At 80 mM NaCl, intracellular CO_2 concentration decreased significantly over the control and K addition improved it significantly, but it did not attain the level observed in the control.

The photosynthetic process can be negatively affected by salinity due most importantly to (i) altered stomatal closure thus limiting CO_2 diffusion to the chloroplasts, (ii) modification in the structure and function of photosynthetic organelles (iii) alterations in light reactions (leading to increase in electron flux to O_2 resulting consequently in an accumulation of oxidative stressors, including TBARS, in the chloroplast), and/or (iv) reduced rate of transport of assimilates and intermediary compounds to the metabolic sinks, (v) efficiency of the root system in limiting the transport of ions to shoots [5,13,34,36,37].

The deleterious effects of salinity on plant growth and physiology are associated with a number of other important factors, including nutrient imbalance, the toxic effects of ions (mainly Na^+ and Cl^-) and/or the oxidative damage resulting from imbalance between production of reactive oxygen species (ROS) and the antioxidant defence [7].

Plant tolerance to salinity stress may be due to ion homeostasis, osmotic adjustment, and a synchronous action of different components of the antioxidant defence system [6,38]. In addition, increasing evidence suggests that maintenance of the K status of plants plays a critical role in increasing both crop productivity and resistance to environmental stress, including salinity [13,37]. K has been shown to play a significant role in a large range of metabolic functions in plants, including growth, photosynthesis, enzyme activation and osmotic potential [27] in addition to lowering the ROS production by reducing activity of NAD(P)H oxidases and maintaining photosynthetic electron transport [13]. In this context, we have shown that the addition of K to the NaCl-treated plants proved to be effective in protecting *B. campestris* plants against NaCl-caused oxidative stress by decreasing Na^+ and Cl^- content and increasing K^+ in leaves. The antagonistic effect of K^+ and Na^+ on uptake of each other is well documented and reported in both saline and sodic conditions [10,27,39,40]. Added K also improved AsA and GSH contents, and further enhanced the activity of APX and GR, whereas the activity of SOD dropped with K-supplementation to the NaCl-treated plants.

In fact, the generation of ROS is an important consequence of a number of stress factors including salinity stress in plants [6]. ROS are metabolised by an efficient antioxidant defence system wherein the enzymatic antioxidants SOD dismutase $O_2^{\cdot-}$ to H_2O_2 , which is further detoxified by APX and/or catalase (data not shown), whereas non-enzymatic antioxidants AsA and GSH have a tight control over the generation and scavenging of ROS [6,39]. AsA-GSH-cycle metabolism is an essential mechanism of plant resistance under stressful conditions [38,41]. Glutathione removes excess H_2O_2 [42] and lipid peroxides keep ROS under control [43]. APX detoxifies H_2O_2 into water using AsA as the major substrate where GSH continuously regenerates AsA from DHA, the oxidized form, via the AsA-GSH cycle. In this process GSH is oxidised into GSSG, which is subsequently recycled by GR [42]. In the present study, K-supplementation to NaCl-treated plants further enhanced the GR activity (maximally at 40 mM NaCl) and thus maintained coordination between the enzymatic (SOD, APX and GR) and non-enzymatic (AsA, GSH) antioxidants. Earlier, increased glutathione content due to sulphur application had been found to protect dry mass and photosynthesis in cadmium-treated mustard (*Brassica campestris*) plants [43]. In addition to other physiological effects of nutrition in plants, glutathione maintains the endogenous level of K^+ in plant tissues and reduces the excess uptake and translocation of undesirable ions including Na^+ and Cl^- [13,14]. K^+ is the most prominent competitor in the uptake of Na^+ under salt stress [44]. K nutrition ameliorated to a great extent the Na^+ and Cl^- - accrued decrease in reducing equivalents viz., AsA and GSH which, in turn, had a tight control over the balance between production and scavenging of H_2O_2 and stabilized the cell membrane

by decreasing TBARS content through coordination with other components of the AsA-GSH cycle. Our findings that K nutrition improves the leaf K^+ and decreases the Na^+ and Cl^- contents confirm those of Qadar and Azam [10], Zheng *et al.* [40,45] and More and Manchanda [39].

5. Conclusions

NaCl stress severely retarded plant growth and photosynthesis, increased the ion toxicity in terms of increasing leaf Na^+ and Cl^- contents, induced lipid peroxidation (TBARS), caused oxidative stress (H_2O_2) and modulated the plant antioxidant system to a great extent. K nutrition exhibited an antagonistic effect on Na^+ and Cl^- accumulation, controlled the SOD and APX activities required for the removal of H_2O_2 and TBARS, and up-regulated GR activity to improve the cellular reducing equivalents AsA and GSH in order to protect plants from NaCl-caused oxidative stress. A protocol for the application of an optimal level of K to the NaCl-affected soil may be developed to achieve increased crop productivity in a sustainable way.

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References

- [1] Martinez-Beltran J., Manzur C.L., Overview of salinity problems in the world and FAO strategies to address the problem, Proc. Intl. Sal. For., Riverside, California, 2005
- [2] Ashraf M., Harris P.J.C., Potential biochemical indicators of salinity tolerance in plants, Plant Sci., 2004, 166, 3-16
- [3] Garthwaite A.J., Bothmer R.V., Colmer T.D., Salt tolerance in wild *Hordeum* species is associated with restricted entry of Na^+ and Cl^- into the shoots, J. Exp. Bot., 2005, 56, 2365-2378
- [4] Cuin T.A., Shabala S., Compatible solutes reduce ROS-induced potassium efflux in Arabidopsis roots, Plant Cell Env., 2007, 30, 875-885
- [5] Munns R., James R.A., Läuchli A., Approaches to increasing the salt tolerance of wheat and other cereals, J. Exp. Bot., 2006, 57, 1025-1043
- [6] Khan N.A., Nazar R., Anjum N.A. Growth, photosynthesis and antioxidant metabolism in mustard (*Brassica juncea* L.) cultivars differing in ATP-sulfurylase activity under salinity stress, Sci. Hortic., 2009, 122, 455-460
- [7] Vital S.A., Fowler R.W., Virgen A., Gossett D.R., Banks S.W., Rodriguez J., Opposing roles for superoxide and nitric oxide in the NaCl stress-induced upregulation of antioxidant enzyme activity in cotton callus tissue, Env. Exp. Bot., 2008, 62, 60-68

- [8] Foyer C.H., Descourvires P., Kunert K.J., Protection against oxygen radicals: An important defence mechanism studied in transgenic plants, *Plant Cell Env.*, 1994, 17, 507-523
- [9] Qadar A., Alleviation of sodicity stress on rice genotypes by phosphorus fertilization, *Plant Soil*, 1998, 203, 269-277
- [10] Qadar A., Zake M.A. Selecting rice genotypes tolerant to zinc deficiency and sodicity : differences in concentration of major cations and sodium/potassium ratio in leaves, *J. Plant Nutr.*, 2007, 30, 2061-2076
- [11] Grattan S.R., Grieve C.M., Salinity nutrient relations in horticultural crops, *Sci. Hortic.*, 1999, 78, 127-157
- [12] Cherel L., Regulation of K⁺ channel activities in plants: from physiological to molecular aspects, *J. Exp. Bot.*, 2004, 55, 337-51
- [13] Cakmak I., The role of potassium in alleviating detrimental effects of abiotic stresses in plants, *J. Plant Nutr. Soil Sci.*, 2005, 168, 521-530
- [14] Collins R.P., Harris P.J.C., Bateman, M.J., Henderson J., Effect of calcium and potassium nutrition on yield, ion content, and salt tolerance of *Brassica campestris* (rapa), *J. Plant Nutr.*, 2008, 31, 1461-1481
- [15] Diva I., Ameliorative effect of potassium on growth and yield of *Brassica campestris* L and *Vigna radiata* (L.) Wilczek under the influence of salinity, heavy metal and water stress, PhD Thesis, 2007, Jamia Hamdard, New Delhi, India
- [16] Hewitt E.J. Sand and water cultural methods used in the study of plant nutrition, *Comm. Agric. Bur.*, England, 1966, p. 56
- [17] Cakmak I., Horst W.J., Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, peroxidase activities in root tips of soybean (*Glycine max* L.), *Physiol. Plant*, 1991, 83, 463-468
- [18] Okuda T., Masuda Y., Yamanka A., Sagisaka S., Abrupt increase in the level of hydrogen peroxide in leaves of winter wheat is caused by cold treatment, *Plant Physiol.*, 1991, 97, 1265-1267
- [19] Anderson M.E., Determination of glutathione and glutathione disulfides in biological samples, *Meth. Enzymol.*, 1985, 113, 548-570
- [20] Law M.E., Charles S.A., Halliwell B., Glutathione and ascorbic acid in spinach (*Spinacia oleracea*) chloroplasts: the effect of hydrogen peroxide and of paraquat, *Biochem. J.*, 1983, 210, 899-903
- [21] Dhindsa R.H., Plumb-Dhindsa P., Thorpe T.A., Leaf senescence correlated with increased level of membrane permeability, lipid peroxidation and decreased level of SOD and CAT, *J. Exp. Bot.*, 1981, 32, 93-101
- [22] Nakano Y., Asada K., Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts, *Plant Cell Physiol.*, 1981, 22, 867-880
- [23] Foyer C.H., Halliwell B., The presence of glutathione and glutathione reductase in chloroplasts: a proposed role in ascorbic acid metabolism, *Planta*, 1976, 133, 21-25
- [24] Lindner R.C., Rapid analytical method for some of the more common organic substances of plant and soil, *Plant Physiol.*, 1944, 19, 76-84
- [25] Munns R., Tester M., Mechanisms of salinity tolerance, *Annu. Rev. Plant Biol.*, 2008, 59, 651-681
- [26] Vassilev A., Yordanov I., Tsonev T., Physiological responses of two barley cultivars to soil pollution with cadmium, *Env. Pollut.*, 1998, 100, 1-7
- [27] Marschner H., Mineral Nutrition of Higher Plants, 2nd Edn. Academic Press, London, 2002
- [28] Arshi A., Abidin M.Z., Iqbal M., Ameliorative effects of CaCl₂ on growth, ionic relations and proline content of senna under salinity stress, *J. Plant Nutr.*, 2005, 28, 101-125
- [29] Chartzoulakis K., Klapaki G., Response of two green house pepper hybrids to NaCl salinity during different growth stages, *Sci. Hortic.*, 2000, 86, 247-260
- [30] Muñoz-Ramos J.J., Guzmán M., Castellanos J.Z., Salinity in vegetative and reproductive growth of pepper, [Salinidad sódica en el desarrollo vegetativo y reproductivo del pimiento], *Terr. LatinoAm.*, 2004, 22, 187-196 (in Spanish)
- [31] Saied S., Keutgen A.J., Noga G., Effects of NaCl stress on leaf growth, photosynthesis and ionic contents of strawberry cvs 'Elsanta2 and 'Korona', *Acta Hortic.*, 2003, 609, 67-73
- [32] Taleisnik E., Rodríguez A.A., Bustos D., Erdei L., Ortega L., Senn M.E., Leaf expansion in grasses under salt stress, *J. Plant Physiol.*, 2009, 166, 1123-1140
- [33] Elkhatab H.A., Elkhatab E.A., Khalaf Allah A.M., El-Sharkawy A.M., Yield response of salt-stressed potato to potassium fertilization: A preliminary mathematical model, *J. Plant Nutr.*, 2004, 27, 111-122
- [34] Chaves M.M., Flexas J., Pinheiro C., Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell, *Ann. Bot.*, 2009, 103, 551-560
- [35] Chow W.S., Ball M.C., Anderson J.M., Growth and photosynthetic responses of spinach to salinity: Implications of K nutrition for salt tolerance, *Aust. J. Plant Physiol.*, 1990, 17, 563-578
- [36] Centritto M., Loreto F., Chartzoulakis K., The use of low [CO₂] to estimate diffusional and non-

- diffusional limitations of photosynthetic capacity of salt-stressed olive saplings, *Plant Cell Environ.*, 2003, 26, 585-94
- [37] Degl' Innocenti E., Hafsi C., Guidi L., Navari-Izzo F., The effect of salinity on photosynthetic activity in potassium-deficient barley species, *J. Plant Physiol.*, 2009, 166, 1968-1981
- [38] Anjum N.A., Umar S., Iqbal M., Khan N.A., Cadmium causes oxidative stress in moongbean [*Vigna radiata* (L.) Wilczek] by affecting antioxidant enzyme systems and ascorbate-glutathione cycle metabolism. *Russ. J. Plant Physiol.*, 2011, 58, 92-99
- [39] More R.P., Manchanda H.R., Influence of phosphorus on the tolerance of table pea to chloride and sulphate salinity in a sandy soil, *Arid Soil Res. Rehab.*, 1992, 6, 41-52
- [40] Zheng Y., Jia A., Ning T., Xu J., Li Z., Jiang G., Potassium nitrate application alleviates sodium chloride stress in winter wheat cultivars differing in salt tolerance, *J. Plant Physiol.*, 2008, 165, 1455-1465
- [41] Szalai G., Kellos T., Galiba G., Kocsy G., Glutathione as an antioxidant and regulatory molecule in plants under abiotic stress conditions, *J. Plant Growth Regul.*, 2009, 28, 66-80
- [42] Noctor G., Arisi A.C.M., Jouanin L., Kunert K.J., Rennenberg H., Foyer C.H., Glutathione: biosynthesis, metabolism and relationship to stress tolerance explored in transgenic plants, *J. Exp. Bot.*, 1998, 49, 623-647
- [43] Rausch T., Gromes R., Liedschulle V., Muller I., Bogs J., Galovic V., et al ., Novel insight into the regulation of GSH biosynthesis in higher plants, *Plant Biol.*, 2007, 9, 565-572
- [44] Marschner H., *Mineral Nutrition of Higher Plants*, Academic Press, London, 1995
- [45] Zheng Y., Xu X., Simmons M., Li Z., Responses of physiological parameters, grain yield and grain quality to foliar application of potassium nitrate in two contrasting winter wheat cultivars under salinity stress, *J. Plant Nutr. Soil Sci.*, 2010, 173, 444-452